BIOBEHAVIORAL RESPONSES TO CONDITIONED FEAR AND RESTRAINT STRESS IN BORDERLINE HYPERTENSIVE RATS: EFFECTS OF NEONATAL HANDLING

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ABSTRACT

The purpose of this experiment was to determine the effects of an early environmental manipulation, handling (H), on cardiovascular (CV) reactivity, freezing behavior and corticosterone (CORT) responses to fear conditioning in the BHR, which is genetically susceptible to environmental stressors. H subjects were separated from the nest for 15 mins/day on post-natal days 1-14, while non-handled (NH) controls remained in the home cage. Adult BHR from each group were implanted with a femoral arterial catheter for mean arterial pressure (MAP) recording and blood sampling. Two days later, subjects were exposed to the fear conditioning procedure. Subjects were returned to the chamber the next day for 10 min during which time freezing behavior, CV responses and CORT measurements were taken. H subjects displayed significantly more freezing behavior compared to NH (92%±2.2 vs. 80.7%±5.7, $p < .05$). Although resting MAP did not differ between groups, H subjects had increased MAP reactivity when returned to the fear conditioning chamber ($p < .01$). Finally, H subjects had significantly lower CORT levels at the end of the 10 min test period (173±8 ng/ml vs. 217.7±22.2 ng/ml, $p < .05$). In a separate experiment, H subjects showed reduced CORT levels in response to acute restraint stress. These results indicate that neonatal H produces enhanced biobehavioral responses to fear conditioning in BHR and may suggest a useful model with which to study the interaction of genetics, emotionality, and heart disease.

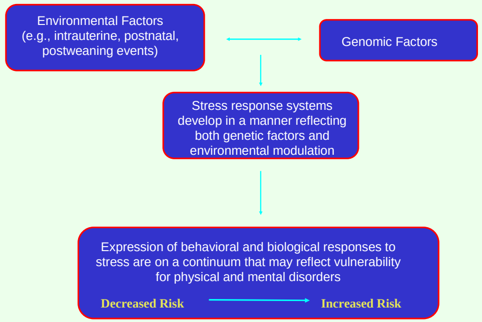
INTRODUCTION

An important area in developmental psychobiology is the examination of how early life events affect the development of offspring. In humans, studies summarized by Francis, et al. (1999) have shown that children raised in environments characterized by neglect or abuse have higher than average incidence of physical disease (e.g., cancer, heart disease) and psychopathology (e.g., anxiety, depression).In order to study how early life events shape biology, researchers have turned to animal models which offer more experimental control and in which developmental questions can be more readily answered.

Investigations in rodents typically attempt to alter early life experience by manipulating the mother and pup interactions. For example, studies have shown that separation of the pups from the nest for an extended period of time significantly increases stress reactivity of the offspring as adults (Francis, 1999). Conversely, brief periods of separation, known as handling, has a protective effect on stress reactivity in adulthood (Liu, 1997; Francis, 1999). The handling behavior is observed when a mother is separated from her neonates for a short time period (about 15 minutes). Upon reintroduction to the home cage, the mother shows increased levels of licking and grooming behaviors with her pups, as well as arched back nursing, in comparison to those left undisturbed. One of the primary consequences of this interaction is decreased of responsiveness of the hypothalamic-pituitary-adrenal (HPA) axis to physical and emotional stressors. This effect is often referred to "stress immunization" since the adult offspring display dampened biological responses to stress (Liu, 1997).

An important role of the HPA axis is to stimulate the release of corticosterone from the adrenal cortex. This system is governed in part by a negative feedback loop whereby the brain monitors circulating levels of corticosterone and adjusts further output to maintain physiologically appropriate levels. Recent studies have shown that postnatally handled rats show enhanced glucocorticoid negative feedback sensitivity compared to nonhandled rats. This increased sensitivity is due to an increase in glucocorticoid receptor expression in the hippocampus. Since the hippocampus is critical for memory, it has been suggested that the alteration in glucocorticoid receptor expression may lead to enhanced memory. Indeed, a recent study demonstrated that neonatally handles rats had increased freezing behavior in a contextual fear conditioning paradigm, suggesting increased memory ability (Beane, 2001).

In addition to early experiential factors, sensitivity to stress in adulthood is also influenced by genetic factors. The borderline hypertensive rat (BHR) is the offspring of one hyper- and one normotensive parent and therefore shows exaggerated reactivity to environmental stressors. rats are sensitive to environmental stressors (Sanders, 1992). The purpose of this study was to examine the interaction between early life experience and genetic background in influencing the stress response. In the first experiment, we sought to determine the effects of handling on cardiovascular reactivity, freezing behavior, and corticosterone levels in response to a fear conditioning procedure (emotional memory). We also asked whether the corticosterone response to an acute physical stressor was affected by neonatal handling.



METHOD

- Borderline hypertensive rats (BHR) were generated in our animal facility and were culled 8 pups on the day of birth.
- Handling was conducted on post-natal days 1-14 by placing each pup in a small isolation chamber for 15 minutes each day. Control litters were left undisturbed.
- Animals were implanted with a femoral arterial catheter for recording blood pressure and heart rate, and obtaining a plasma sample.
- Contextual fear conditioned was carried out in subjects 48h after surgery. In this procedure, animals were placed in a small chamber with dim light, background noise, and a peppermint odor and allowed to explore for 30 sec at which time they received a 1 mA footshock of .2 sec in duration. They remained in the chamber for an additional 30 sec before returned to the home cage.
- Subjects were returned to the fear conditioning chamber 24 h later. Freezing behavior, blood pressure and heart rate were recorded, and a blood sample was taken for corticosterone measurements.
- In another experiment, corticosterone responses to acute restraint stress were determined in separate subjects from each group. Plasma samples were taken during baseline prior to placing subjects in a Plexiglas restrainer. Additional samples were taken at 30 and 60 min after being placed in the restrainer. After 60 min, subjects were returned to the home cage and additional samples were taken at 90 and 120 min following the initiation of the stressor.

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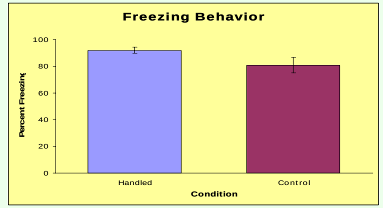

Figure 1. When re-exposed to the fear conditioning context, handled subjects (n=16) displayed significantly more freezing behavior compared to non-handled controls (N=13); t (27) = 1.97, $p < .05$.

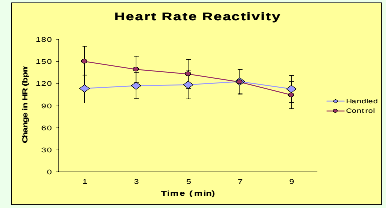

Figure 3. There were no significant effects found with respect to heart rate reactivity between handled (n = 9) and control (n=7) subjects.

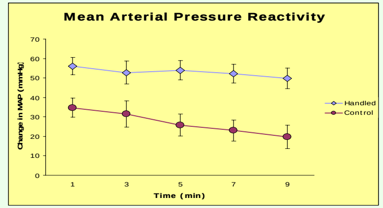

Figure 2. Neonatal handling (n=9) produced significantly greater change in blood pressure from baseline compared to controls (n=7). ANOVA revealed a significant main effects of time $F(4,56) = 7.99$, $p < .0001$ and condition $F(1,14) = 12.89$, $p < .003$, and a marginally significant interaction of time x condition $F(4,56) = 2.20$, $p < .081$.

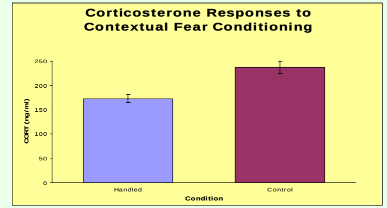

Figure 4. Corticosterone levels taken 10 min after re-exposure to the fear conditioning chamber were significantly lower in handled (n=8) compared to control (n=6) subjects; t (12) = 2.10, p < .057.

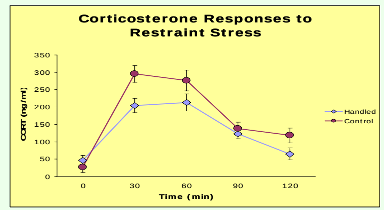

Figure 5. This graph depicts corticosterone levels taken during a baseline period (time = 0), twice during restraint stress (time = 30 and 60 min), and twice after return to the home cage (time = 90 and 120 min). Repeated measures ANOVA revealed a significant main effect of time, F (4,40) = 61.78, $p < .0001$ and condition F (1,10) = 4.62, $p < .057$ and significant interaction of time x condition F (4, 40) = 3.23, $p < .022$.

SUMMARY AND CONCLUSIONS

The main findings of this study are that BHR exposed to neonatal handling during the first two weeks of life react, as adults, to fear conditioning differently than control subjects. Specifically, handled subjects show increased freezing behavior and blood pressure reactivity when tested 24h after fear conditioning, suggesting a heightened emotional response. Moreover, handled subjects have lower corticosterone levels when re-exposed to the fear conditioning context. Based on other studies, a reasonable interpretation of these findings is that the enhanced glucocorticoid receptor expression in the hippocampus that results from handling provides these subject with a better memory for the fear producing event that occurred in the chamber, as well as a more sensitive negative feedback system to modulate corticosterone levels. In the second experiment, we found that handling results in reduced corticosterone to 60 min of restraint stress. Thus, corticosterone responses in handled subjects are reduced in response to both emotional and physical stressors.

The results of this experiment suggests that both genetics and experience can influence an organism's response to fearful or stressful situations. Handled subjects responded to re-exposure to the fear conditioning chamber with increased freezing behavior (a classic response to fear) and decreased corticosterone levels, both of which are consistent with previous research. However, these subjects also had significantly higher blood pressure during this test compared to non-handled subjects. These observations suggest that this model may represent a useful approach for studying the interaction between family history, emotional stress, and risk for heart disease.